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ADDRESS TO: **Assistant Commissioner for Patents** **Box Patent Application** **Washington, D.C. 20231**	Attorney Docket No. MBHB00-025 First Named Inventor Choong et al. Express Mail No. EL028731742US Total Pages 41

APPLICATION ELEMENTS

1. ☒ Transmittal Form with Fee
2. ☒ Specification (including claims and abstract) [Total Pages 31]
3. ☒ Drawings [Total Sheets 4]
4. ☒ Oath or Declaration [Total Pages 3]
   a. ☒ Newly executed
   b. ☐ Copy from prior application **[Note Boxes 5 and 17 below]**
      i. ☐ Deletion of Inventor(s) Signed statement attached deleting inventor(s) named in the prior application
5. ☐ Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
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ACCOMPANYING APPLICATION PARTS

8. ☐ Assignment Papers
9. ☒ Power of Attorney
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11. ☐ Information Disclosure Statement (IDS)
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UTILITY PATENT APPLICATION TRANSMITTAL

Attorney Docket No. MBHB00-25

APPLICATION FEES				
BASIC FEE				$ 690.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total Claims	132 -20=	112	x $18.00	$ 2016.00
Independent Claims	4 - 3=	1	x $78.00	$ 78.00
☒ Multiple Dependent Claims(s) if applicable			+$270.00	$ 270.00
			Total of above calculations =	$ 3054.00.00
			Reduction by 50% for filing by small entity =	$
☐ Assignment fee if applicable			+ $40.00	$ 00
			TOTAL =	$ 3054.00

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23. USPTO CUSTOMER NUMBER

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Signature	
Date	August 31, 2000

APPLICATION FOR UNITED STATES LETTERS PATENT
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 00,025)

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Addressable Array for High Density Electrical and Electrochemical Detection of Biomolecules

ADDRESSABLE ARRAY FOR HIGH DENSITY ELECTRICAL AND ELECTROCHEMICAL DETECTION OF BIOMOLECULES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the detection of molecular interactions between biological molecules. Specifically, the invention relates to the electrical or electrochemical detection of molecular interactions on biochip arrays. More specifically, the invention relates to an apparatus for the electrical or electrochemical detection of molecular interactions comprising a column-and-row addressable biochip array and methods of use thereof. The apparatus and methods of the invention can be used to detect molecular interactions such as nucleic acid hybridization or peptide binding.

2. Background of the Invention

A number of commonly-utilized biological applications, including for example, diagnoses of genetic disease, analyses of sequence polymorphisms, analyses of gene expression, and studies of receptor-ligand interactions, rely on the ability of analytical technologies to readily detect events related to the interaction between biological molecules (herein after, "biomolecules"). These detection technologies have traditionally utilized fluorescent compounds or radioactive isotopes to monitor such interactions. For example, Potyrailo *et al.*, 1998, *Anal. Chem.* 70: 3419-25, describe an apparatus and method for detecting interactions between immobilized fluorescently-labeled aptamers and peptides. There are, however, significant disadvantages associated with the use of radioactive or fluorescent labels to track interactions between biomolecules, including heightened health risks and increased experimental cost and complexity.

Methods for electrical or electrochemical detection of molecular interactions between biomolecules have provided an attractive alternative to detection techniques relying on radioactive or fluorescent labels. Electrical and electrochemical detection techniques are based on the detection of alterations in the electrical properties of an electrode arising from interactions between one group of molecules attached to the surface of an electrode (often referred to as

"probe" molecules) and another set of molecules present in a reaction mixture (often referred to as "target" molecules) contacted with the electrode. Methods and devices related to electrical or electrochemical detection of biomolecules are disclosed in U.S. Patent Nos. 4,072,576, 4,098,645, 4,414,323, 4,840,893, 5,164,319, 5,187,096, 5,194,133, 5,312,527, 5,532,128, 5,591,578, 5,653,939, 5,670,322, 5,705,348, 5,770,369, 5,780,234, 5,824,473, 5,891,630, 6,017,696 and International Application, Pub. No. WO 97/01646.

Electrical or electrochemical detection eliminates many of the disadvantages inherent in use of radioactive or fluorescent labels to detect interactions between the probe and target molecules. This process offers, for example, a detection technique that is safe, inexpensive, and sensitive, and is not burdened with complex and onerous regulatory requirements.

The development of microfabricated arrays (microarrays) of biomolecules has led to further improvements on traditional analytical techniques for the detection of molecular interactions between biomolecules. Microarrays of biomolecules (*e.g.*, oligonucleotides, nucleic acids, proteins, peptides, or antibodies) have utility in a wide variety of applications in which molecular interactions between target molecules in a reaction mixture and large numbers of distinct probe molecules bound to defined regions of a substrate can be simultaneously assayed using electrical, optical, or radioactive detection strategies. Microarrays, therefore, satisfy the demand for inexpensive, high-throughput detection of biomolecular interactions.

Although biochip arrays for the electrochemical detection of molecular interactions between biomolecules have been proposed in the prior art, these devices have significant disadvantages. For example, the device disclosed by Egger *et al.* in U.S. Patent Nos. 5,670,322 and 5,532,128 cannot be made column-and-row (or "x-y") addressable, thus limiting the density of the test sites in the array and the usefulness of the apparatus. In U.S. Patent No. 5,653,939, Hollis *et al.* disclose an x-y addressable array wherein a solid supporting substrate comprises a plurality of test sites in electrochemical contact with a set of orthogonally oriented electrodes. However, Hollis *et al.* does not provide an apparatus for efficient electrochemical detection of molecular interactions on porous, polymeric pads. Furthermore, Hollis *et al.* does not provide an apparatus having interdigitated electrodes.

Thus, there remains a need in the art to develop more efficient devices and methods for the detection of molecular interactions between biomolecules. In particular, there remains a need in the art for more efficient devices and methods for the electrical or electrochemical detection of

molecular interactions. More particularly, there remains a need in the art to develop column-and-row addressable biochip arrays for the electrical or electrochemical detection of molecular interactions that can be easily and cost-effectively fabricated, and that reduce the cost of performing various analyses, while increasing the effectiveness and utility thereof. The development of such devices, and methods for their use, would have wide application in the medical, genetic, and molecular biological arts.

SUMMARY OF THE INVENTION

The present invention provides an apparatus and methods for the electrical or electrochemical detection of molecular interactions between biological molecules. Specifically, the invention provides an apparatus for the electrical or electrochemical detection of molecular interactions between biological molecules that comprises a column-and-row addressable biochip array. The apparatus and methods of the invention can be used to detect molecular interactions such as nucleic acid hybridization or peptide binding.

One apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a plurality of porous, polymeric pads in contact with the supporting substrate at the test sites; a set of input electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each output electrode is arranged to address a subset of the test sites, and wherein each output electrode is in electrochemical contact with an input electrode; a plurality of linker moieties in contact with the porous, polymeric pads at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the porous polymeric pads, input electrodes, output electrodes, linker moieties, and probe molecules.

Another apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a plurality of porous, polymeric pads in contact with the supporting

substrate at the test sites; a set of input electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each output electrode is arranged to address a subset of the test sites, and wherein each output electrode is in electrochemical contact with an input electrode; a plurality of linker moieties in contact with the porous, polymeric pads at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; at least one reference electrode in electrochemical contact with the input and output electrodes; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the porous polymeric pads, input electrodes, output electrodes, linker moieties, and probe molecules.

Still another apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a set of input electrodes in contact with the supporting substrate, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the supporting substrate at the test sites, wherein each output electrode is arranged to address a subset of the test sites, each output electrode is in electrochemical contact with an input electrode, and the output electrodes and input electrodes are interdigitated at the test site; a plurality of linker moieties in contact with either the input electrodes, the output electrodes, or both the input electrodes and output electrodes at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the input electrodes, output electrodes, linker moieties, and probe molecules.

Still another apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a set of input electrodes in contact with the supporting substrate, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the supporting substrate at the test sites, wherein each output electrode is arranged to address a subset of the test sites, each output electrode is in electrochemical contact with an input electrode, and the output electrodes and input electrodes

are interdigitated at the test site; a plurality of linker moieties in contact with either the input electrodes, the output electrodes, or both the input electrodes and output electrodes at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; at least one reference electrode in electrochemical contact with the input and output electrodes; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the input electrodes, output electrodes, linker moieties, reference electrode, and probe molecules.

The apparatus of the present invention may further comprise a plurality of wells wherein each well encompasses a porous, polymeric pad, wherein a plurality of probe molecules is immobilized to linker moieties that are in contact with the porous, polymeric pad; an input electrode, and an output electrode. Preferably, the probe molecules in any particular well are identical to each other, while each well comprises probe molecules unique to that well.

The present invention provides methods employing the apparatus that are useful for electrical or electrochemical detection of molecular interactions between probe molecules immobilized to linker moieties in contact with porous, polymeric pads and target molecules in a sample solution. In one method of the present invention, a first electrical signal is applied at an input electrode in contact with a first set of porous, polymeric pads, wherein the first set of porous, polymeric pads comprises the porous, polymeric pad at the specific test site; and the first electrical signal is then detected at an output electrode in contact with a second set of porous, polymeric pads, wherein the second set of porous, polymeric pads comprises the porous, polymeric pad at the specific test site. Thereafter, the first and second sets of porous, polymeric pads are exposed to a sample mixture containing a particular target molecule; a second electrical signal is applied at an input electrode in contact with the first set of porous, polymeric pads; and the second electrical signal is detected at an output electrode in contact with the second set of porous, polymeric pads. The first and second electrical signals are compared, and molecular interactions between immobilized probe molecules and target molecules in the sample mixture are detected by determining that the first electrical signal is different from the second electrical signal.

In some embodiments of the methods of the present invention, target molecules in a sample mixture are labeled with an electrochemically-active reporter molecule prior to exposing the first and second sets of porous, polymeric pads to the sample mixture.

The x-y addressable bioarrays of the present invention can be employed for both electrical and electrochemical detection, thus permitting a wider number of analyses to be performed on these devices. The x-y addressing scheme simplifies and reduces the number of electrode interconnections required, thus permitting the bioarrays of the present invention to be more cost-effectively fabricated. The three-dimensional design of the input and output electrodes increases the surface area of the electrodes, thereby increasing the efficiency by which the devices can be used to electrically and electrochemically detect molecular interactions between biomolecules. Furthermore, in those devices of the invention in which the input and output electrodes are interdigitated, such interdigitation allows one with skill in the art to fabricate a more efficient device for a particular electrical or electrochemical detection scheme by altering the distance between the input and output electrodes.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a schematic representation of a top view of one embodiment of the x-y addressable biochip array of the present invention.

Figures 2A and 2B illustrate schematic representations of two cross-section views of one embodiment of the x-y addressable biochip array of the present invention.

Figure 3 illustrates a schematic representation of a top view of one embodiment of the x-y addressable biochip array of the present invention.

Figures 4A and 4B illustrate schematic representations of two cross-section views of one embodiment of the x-y addressable biochip array of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The apparatus and methods of the present invention are useful for the electrical or electrochemical detection of molecular interactions between target molecules in a sample mixture and probe molecules immobilized on a biochip array. Specifically, the invention provides an apparatus for the electrical or electrochemical detection of molecular interactions between biological molecules that comprises a column-and-row addressable biochip array.

As used herein, the term "array" refers to an ordered spatial arrangement, particularly an arrangement of immobilized biomolecules at a plurality of test sites.

As used herein, the term "addressable array" refers to an array wherein the individual test sites have precisely defined x- and y-coordinates, so that a given test site at a particular position in the array can be identified.

As used herein, the terms "probe" and "biomolecular probe" refer to a biomolecule used to detect a complementary biomolecule (referred to herein as a target molecule). Examples include antigens that detect antibodies, oligonucleotides that detect complimentary oligonucleotides, and ligands that detect receptors. Preferred probe molecules include nucleic acids, oligonucleotides, peptides, ligands, antibodies, and antigens; oligonucleotides are the most preferred probe species.

As used herein, the terms "microarray," "biochip" and "biochip array" refer to an ordered spatial arrangement of immobilized biomolecular probes arrayed at test sites on a solid supporting substrate. Biochips, as used in the art, encompass substrates containing arrays or microarrays, preferably ordered arrays and most preferably ordered, addressable arrays, of biomolecules that comprise one member of a biological binding pair. Typically, such arrays are oligonucleotide arrays comprising a nucleotide sequence that is complementary to at least one sequence that may be or is expected to be present in a biological sample. Alternatively, proteins, peptides or other small molecules can be arrayed in such biochips for performing, *inter alia*, immunological analyses (wherein the arrayed molecules are antigens) or assaying biological receptors (wherein the arrayed molecules are ligands, agonists or antagonists of said receptors).

As used herein, the term "test site" refers to a predefined region on a substrate to which a subgroup of the array's probe molecules are immobilized via linker moieties that are in contact

with a porous, polymeric pad or an input and/or output electrode. The test site may have any convenient shape, *e.g.*, circular, rectangular, elliptical, or wedge-shaped. In preferred embodiments of the apparatus of the present invention, the test sites have an area of about 1 cm^2. In more preferred embodiments, the test sites have an area of less than 1 mm^2, less than 0.5 mm^2, less than about 10,000 μm^2, or less than 100 μm^2. Probe molecules may be immobilized by first placing the linker moieties in contact with a porous, polymeric pad or an input and/or output electrode and then attaching the probe molecules to the porous, polymeric pad or input and/or output electrode. Alternatively, probe molecules may immobilized by first mixing the probe molecules with the linker moieties and then placing the probe/linker moiety mixture in contact with a porous, polymeric pad or an input and/or output electrode. Alternatively, probe molecules may be immobilized by first mixing the probe molecules with the linker moieties and porous, polymeric pads constituents and then polymerizing this mixture on the support substrate.

As used herein, the term "input electrode" refers to an electrode that can be used to apply an electrical signal to a particular test site. In some embodiments, the electrical signal is applied to the input electrode using a multiplexer. As used herein, the term "multiplexer" refers to a device that allows electrical signals to be selectively applied to two or more input electrodes.

As used herein, the term "output electrode" refers to an electrode that can be used to detect an electrical signal at a particular test site. In some embodiments, the electrical signal is detected using a demultiplexer. As used herein, the term "demultiplexer" refers to a device that allows electrical signals from two or more output electrodes to be selectively detected at an electrical signal detection device.

As used herein, the term "reference electrode" refers to an electrode that can be used in assays where an estimate or determination of the number or concentration of target molecules in a sample solution is desired.

Device embodiments of the invention are useful for either electrical or electrochemical detection of interactions between biomolecules. As used herein, the term "electrochemical detection" is intended to encompass methods based on oxidation/reduction (redox) processes induced by electron transfer between electrodes, most preferably mediated by an electrochemical reporter group attached to the probe moiety, the target moiety, or both. As used herein, the term "electrical detection" is intended to encompass methods that rely on impedance changes (such as

resistance, capacitance and inductance) due to differences in electrical state occupancy in the biomolecules in the bound and unbound conformations.

In one embodiment, the apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a plurality of porous, polymeric pads in contact with the supporting substrate at the test sites; a set of input electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each output electrode is arranged to address a subset of the test sites, and wherein each output electrode is in electrochemical contact with an input electrode; a plurality of linker moieties in contact with the porous, polymeric pads at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the porous polymeric pads, input electrodes, output electrodes, linker moieties, and probe molecules.

In another embodiment, the apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a plurality of porous, polymeric pads in contact with the supporting substrate at the test sites; a set of input electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each output electrode is arranged to address a subset of the test sites, and wherein each output electrode is in electrochemical contact with an input electrode; a plurality of linker moieties in contact with the porous, polymeric pads at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; at least one reference electrode in electrochemical contact with the input and output electrodes; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the porous polymeric pads, input electrodes, output electrodes, linker moieties, and probe molecules.

In still another embodiment, the apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a set of input electrodes in contact with the

supporting substrate, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the supporting substrate at the test sites, wherein each output electrode is arranged to address a subset of the test sites, each output electrode is in electrochemical contact with an input electrode, and the output electrodes and input electrodes are interdigitated at the test site; a plurality of linker moieties in contact with either the input electrodes, the output electrodes, or both the input electrodes and output electrodes at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the input electrodes, output electrodes, linker moieties, and probe molecules.

In still another embodiment, the apparatus of the present invention comprises a supporting substrate comprising an array of test sites; a set of input electrodes in contact with the supporting substrate, wherein each input electrode is arranged to address a subset of the test sites; a set of output electrodes in contact with the supporting substrate at the test sites, wherein each output electrode is arranged to address a subset of the test sites, each output electrode is in electrochemical contact with an input electrode, and the output electrodes and input electrodes are interdigitated at the test site; a plurality of linker moieties in contact with either the input electrodes, the output electrodes, or both the input electrodes and output electrodes at the test sites; a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules; at least one reference electrode in electrochemical contact with the input and output electrodes; a means for producing an electrical signal at each input electrode; a means for detecting changes in the electrical signal at each output electrode; and an electrolyte solution in contact with the input electrodes, output electrodes, linker moieties, reference electrode, and probe molecules.

The preferred embodiments of the present invention and its advantages over previously investigated electronic or electrochemical detection devices are best understood by referring to Figures 1-4. Like numerals have been used in the drawings for like and corresponding parts.

Figure 1 illustrates a schematic representation of a top view of one embodiment of the x-y addressable microarray of the present invention. The microarray comprises a supporting substrate **1** comprising a plurality of porous, polymeric pads **2**, the porous, polymeric pads

defining an array of test sites. A set of input electrodes **3** is fabricated within or on top of the supporting substrate **1**, the set of input electrodes **3** being arranged so that each input electrode **3** addresses a subset of test sites. A set of output electrodes **4** is fabricated within or on top of the supporting substrate **1**, the set of output electrodes **4** being arranged so that each output electrode **4** addresses a subset of test sites. In this embodiment, the input electrodes **3** and the output electrodes **4** are embedded within the porous, polymeric pads **2** and the input electrodes **3** and the output electrodes **4** are arranged so that they interdigitate (*see* Figure 1 for one embodiment of electrodes which are interdigitated; other embodiments will be clear to one with skill in the art). Figures 2A and 2B illustrate schematic representations of two cross-section views of one embodiment of the x-y addressable microarray of the present invention. The microarray illustrated in Figures 1 and 2 can be used for the electrical detection of molecular interactions between biomolecules.

Figure 3 illustrates a schematic representation of a top view of another embodiment of the x-y addressable microarray of the present invention. The microarray comprises a supporting substrate **1** comprising a plurality of porous, polymeric pads **2**, the porous, polymeric pads defining an array of test sites. A set of input electrodes **3** is fabricated within or on top of the supporting substrate **1**, the set of input electrodes **3** being arranged so that each input electrode **3** addresses a subset of test sites. A set of output electrodes **4** is fabricated within or on top of the supporting substrate **1**, the set of output electrodes **4** being arranged so that each output electrode **4** addresses a subset of test sites. In this embodiment, the input electrodes **3** and the output electrodes **4** are embedded within the porous, polymeric pads **2** and the input electrodes **3** and the output electrodes **4** are arranged so that they interdigitate (*see* Figure 3). A reference electrode **5** is separated from the input electrodes **3** and output electrodes **4** by either a portion of the supporting substrate **1** (or optionally by an additional insulating layer). Electrochemical contact between the reference electrode 5 and the input electrodes **3** and output electrodes **4** is established through a via **6** fabricated at each test site. Figures 4A and 4B illustrate schematic representations of two cross-section views of one embodiment of the x-y addressable microarray of the present invention. The microarray illustrated in Figures 3 and 4 can be used for the electrical or electrochemical detection of molecular interactions between biomolecules.

By embedding the input and output electrodes in the porous, polymeric pads (as shown in Figures 1 and 3), the surface area of the input and output electrodes in contact with the porous,

polymeric pad can be increased. Similarly, in embodiments in which the input and output electrodes protrude into the test site to contact the sample solution, the surface area of the input and output electrodes in contact with the sample solution is increased. This is advantageous in embodiments in which probe molecules are immobilized via linker moieties to the surface of the input and/or output electrodes. Furthermore, the surface area of the input and output electrodes can be increased by embedding a plurality of projections from a single input and/or output electrode into each porous, polymeric pad (or similarly in some embodiments, into the sample solution at each test site).

The plurality of projections from the input and output electrodes can also be interdigitated (as shown in Figures 1 and 3). By varying the spacing and width of the interdigitated electrodes, the bioarrays of the present invention can be tuned to the specific detection scheme to be employed. For example, for electrical detection schemes (*e.g.*, capacitance), small spacings between the input and output electrodes are desired. Preferably, the spacing between input and output electrodes is less than about 1 micron for the electrical detection devices of the present invention.

The supporting substrate of the apparatus of the invention is advantageously made from any solid material, including but not limited to glass, silicon, silicon nitride, plastic, rubber, fabric, ceramics, printed circuit board, compound semiconductors (*e.g.*, GaAs), or combinations thereof. In preferred embodiments, the supporting substrate of the apparatus of the present invention is composed of silicon or glass. The input, output, and/or reference electrodes of the apparatus of the present invention may be either embedded within or placed in contact with the supporting substrate. The supporting substrate has a surface area between about 0.01 μm^2 and about 5 cm^2 containing from 1 to about 10^8 test sites. In a preferred embodiment, the supporting substrate has a surface area of about 10,000 μm^2 and contains about 10^4 test sites. In preferred embodiments, the test sites are arranged on the supporting substrate so that they are separated by a distance of from about 0.05 μm to 0.5 mm. In more preferred embodiments, the test sites are regularly spaced on the solid supporting substrate with a uniform spacing there between. Preferably, the probe molecules at any particular test site are identical to each other, while each test site comprises probe molecules unique to that test site.

The porous, polymeric pads of the apparatus of the invention are composed of materials including, but not limited to, polyacrylamide gel, agarose gel, polyethylene glycol, cellulose gel,

sol gel, polypyrrole, carbon, carbides, oxides, nitrides, or other porous, polymeric materials known to those with skill in the art. In a preferred embodiment, the porous, polymeric pads comprise polyacrylamide gel.

The input electrodes of the apparatus of the invention comprise conductor substances such as solid or porous foils or films of gold, platinum, silver, copper, titanium, chromium, or aluminum, or metal oxides, metal nitrides, metal carbides, carbon, graphite, conductive plastic (such as polythiophenes, polyanilines, or polypyrroles), metal impregnated polymers, or combinations thereof. In additional embodiments, the input electrodes further comprise substrate and/or insulator substances such as glass, silicon, plastic, rubber, fabric, ceramics, printed circuit board, or combinations thereof.

The output electrodes of the apparatus of the invention comprise conductor substances such as solid or porous foils or films of gold, platinum, silver, copper, titanium, chromium, or aluminum, or metal oxides, metal nitrides, metal carbides, carbon, graphite, conductive plastic (such as polythiophenes, polyanilines, or polypyrroles), metal impregnated polymers, or combinations thereof. In additional embodiments, the output electrodes further comprise substrate and/or insulator substances such as glass, silicon, plastic, rubber, fabric, ceramics, printed circuit board, or combinations thereof.

In some embodiments of the present invention, the linker moieties comprise a conjugated polymer or copolymer film. Such conjugated polymer or copolymer film is composed of materials including, but not limited to, polypyrrole, polythiphene, polyaniline, polyfuran, polypyridine, polycarbazole, polyphenylene, poly(phenylenvinylene), polyfluorene, or polyindole, or their derivatives, copolymers, or combinations thereof. In another preferred embodiment, the linker moieties comprise a neutral pyrrole matrix. In still other embodiments, the linker moieties comprise thiol linkers. In still other embodiments, the linker moieties further comprise streptavidin (and the probe molecules are biotinylated).

The biological molecules of the invention (both probe molecules and target molecules) carry an electrical charge in aqueous solution under appropriate conditions of hydrogen ion concentration and dissolved salts; said conditions are generally determined in part by the composition of the biological sample and the electrolyte solution. Preferably, the probe molecules at any particular test site are identical to each other (*e.g.*, in oligonucleotide embodiments of the apparatus of the present invention, oligonucleotide probe molecules at a test

site all share the same nucleotide sequence), while each test site comprises probe molecules unique to that test site (*e.g.*, in oligonucleotide embodiments of the apparatus of the present invention, the nucleotide sequence of the oligonucleotide probe molecule immobilized at one test site differ from the sequence of the oligonucleotide probe molecules at another test site). In alternative embodiments, the probe molecules at any particular test site are not identical to each other (e.g., in oligonucleotide embodiments of the apparatus of the present invention, oligonucleotide probe molecules at a test site have one of at least two different nucleotide sequences).

In one embodiment of the present invention, the probe molecules are nucleic acids, oligonucleotides, or combinations thereof. Oligonucleotide probe molecules preferably comprise from about 10 to about 100, more preferably from about 10 to about 50, and most preferably from about 15 to about 30, nucleotide residues. Nucleic acid probe molecules comprise from about 10 to about 5000 basepairs, more preferably from about 100 to about 1000 basepairs, and most preferably from about 200 to about 500 basepairs. In a particular embodiment of the present invention, the probe molecules are aptamers (*i.e.*, oligonucleotides capable of interacting with target molecules such as peptides). Oligonucleotide or nucleic acid probe molecules can be immobilized to linker moieties (or immobilized on porous, polymeric pads via linker moieties) using techniques known to those with skill in the art, wherein said immobilization does not interfere with or inhibit the ability of the probe molecules to interact with nucleic acid target molecules in the sample mixture.

In another embodiment of the present invention, the probe molecules of the apparatus comprise proteins or peptides. The protein or peptide probe molecules of the present invention are preferably peptides comprising from about 5 to about 100 amino acids, or preferably antigen-recognizing peptides or polypeptides belonging to the immunoglobulin superfamily. Said peptide or polypeptide probe molecules are immobilized to linker moieties (or immobilized on porous, polymeric pads via linker moieties) using techniques known to those with skill in the art, wherein said immobilization does not interfere with or inhibit the ability of the probe molecules to interact with target molecules in the sample mixture. In one preferred embodiment, the probes are antibodies. The antibodies immobilized to the linker moieties of the apparatus of the invention may be polyclonal or monoclonal antibodies, or F(ab) fragments, F(ab)' fragments,

$F(ab)_2$ fragments, or F_v fragments of polyclonal or monoclonal antibodies, or F(ab) or single chain antibodies selected from *in vitro* libraries.

In still other embodiments of the present invention, the probe molecules comprise a natural products library, a phage display library, or a combinatorial library known to those with skill in the art.

The apparatus of the present invention comprises at least one reference electrode. In the preferred embodiment of the present invention the reference electrode comprises a gold or platinum conductor. In another embodiment, the reference electrode comprises silver/silver chloride. In alternative embodiments, the reference electrode comprises conductor substances such as solid or porous foils or films of silver, copper, titanium, chromium, or aluminum, or metal oxides, metal nitrides, metal carbides, carbon, graphite, conductive plastic (such as polythiophenes, polyanilines, or polypyrroles), metal impregnated polymers, or combinations thereof. In additional embodiments, the reference electrode comprises substrate and/or insulator substances such as glass, silicon, plastic, rubber, fabric, ceramics, printed circuit board, or combinations thereof.

In still other embodiments of the present invention, the apparatus further comprises a plurality of wells wherein each well encompasses a porous, polymeric pad, wherein a plurality of probe molecules is immobilized to linker moieties that are in contact with the porous, polymeric pad; an input electrode; and an output electrode. The term "wells" is used herein in its conventional sense, to describe a portion of the supporting substrate in which the porous, polymeric pad, input electrode, and output electrode are contained in a defined volume; said wells can protrude from the surface of the supporting substrate, or be embedded therein. Preferably, the probe molecules in any particular well are identical to each other, while each well comprises probe molecules unique to that well.

Electrochemical contact between the porous polymeric pads, input electrodes, output electrodes, linker moieties, probe molecules, and reference electrode (when present) is advantageously provided using an electrolyte solution in contact with each of these components. Electrolyte solutions useful in the apparatus and methods of the invention include any electrolyte solution at physiologically-relevant ionic strength (equivalent to about 0.15 M NaCl) and neutral pH. Examples of electrolyte solutions useful with the apparatus and methods of the invention

include but are not limited to phosphate buffered saline, HEPES buffered solutions, and sodium bicarbonate buffered solutions.

In preferred embodiments of the present invention, molecular interactions between immobilized probe molecules and target molecules in a sample mixture are detected by detecting an electrical signal using AC impedance. In other embodiments, such molecular interactions are detected by detecting an electrical signal using an electrical or electrochemical detection method selected from the group consisting of impedance spectroscopy, cyclic voltammetry, AC voltammetry, pulse voltammetry, square wave voltammetry, AC voltammetry, hydrodynamic modulation voltammetry, conductance, potential step method, potentiometric measurements, amperometric measurements, current step method, other steady-state or transient measurement methods, and combinations thereof.

In one embodiment of the apparatus of the present invention, the means for producing electrical impedance at each test electrode is accomplished using a Model 1260 Impedance/Gain-Phase Analyzer with Model 1287 Electrochemical Interface (Solartron Inc., Houston, TX). Other electrical impedance measurement means include, but are not limited to, transient methods using AC signal perturbation superimposed upon a DC potential applied to an electrochemical cell such as AC bridge and AC voltammetry. The measurements can be conducted at any particular frequency that specifically produces electrical signal changes that are readily detected or otherwise determined to be advantageous. Such particular frequencies are advantageously determined by scanning frequencies to ascertain the frequency producing, for example, the largest difference in electrical signal. The means for detecting changes in impedance at each test site electrode as a result of molecular interactions between probe and target molecules can be accomplished by using any of the above-described instruments.

The present invention also provides methods that are useful for the electrochemical detection of molecular interactions between target molecules in a sample solution and probe molecules immobilized to (or attached thereto using) linker moieties bound to, in contact with, or covalently attached to the porous, polymeric pads.

The present invention provides methods employing the apparatus that are useful for electrical or electrochemical detection of molecular interactions between probe molecules immobilized to linker moieties in contact with porous, polymeric pads and target molecules in a sample solution. In one embodiment of the methods of the present invention, a first electrical

signal is applied at an input electrode in contact with a first set of porous, polymeric pads, wherein the first set of porous, polymeric pads comprises the porous, polymeric pad at the specific test site; and the first electrical signal is then detected at an output electrode in contact with a second set of porous, polymeric pads, wherein the second set of porous, polymeric pads comprises the porous, polymeric pad at the specific test site. Thereafter, the first and second sets of porous, polymeric pads are exposed to a sample mixture containing a particular target molecule; a second electrical signal is applied at an input electrode in contact with the first set of porous, polymeric pads; and the second electrical signal is detected at an output electrode in contact with the second set of porous, polymeric pads. The first and second electrical signals are compared, and molecular interactions between immobilized probe molecules and target molecules in the sample mixture are detected by determining that the first electrical signal is different from the second electrical signal.

In some embodiments of the methods of the present invention, detection of molecular interactions between probe and target molecules is accomplished or enhanced by the coupling of an electrochemically-active moiety (termed a "reporter group") to the target molecule. Target molecules labeled with electrochemically-active reporters useful in the methods of the present invention are electrochemically-active, *i.e.*, they are capable of participating in oxidation/reduction (redox) reactions under an applied voltage potential that can be achieved under conditions that are compatible with probe molecules immobilized to linker moieties in contact with the porous, polymeric pads and target molecules in a sample solution. Target molecules labeled with an electrochemically-active moiety useful in the methods of the present invention may be prepared by labeling suitable target molecules with any reporter group having an electrochemically-distinctive property, most preferably a redox potential that can be distinguished from other components of the binding reaction, and that does not interfere with the molecular interaction to be detected. In preferred embodiments of the method of the present invention, target molecules are labeled with electrochemical reporter groups comprising a transition metal complex or an organic redox couple, most preferably containing a transition metal ion that is ruthenium, cobalt, iron, copper, zinc, nickel, magnesium, or osmium; or an organic compound including, but not limited to, methylene blue, viologen, ferrocenes, and quinones.

In other embodiments of the present invention, target molecules are labeled with the following non-limiting examples of electrochemically-active moieties: 1,4-benzoquinone, ferrocene, tetracyanoquinodimethane, N,N,N',N'-tetramethyl-p-phenylenediamine, tetrathiafulvalene, viologen(methyl, aminopropyl viologen), phenylene-diamine, 9-aminoacridine, acridine orange, aclarubicin, daunomycin, doxorubicin, pirarubicin, ethidium bromide, ethidium monoazide, chlortetracycline, tetracycline, minocycline, Hoechst 33258, Hoechst 33342, 7-aminoactinomycin D, Chromomycin A_3, mithramycin A, Vinblastine, Rifampicin, $Os(bipyridine)_2(dipyridophenazine)_2^{+}$, $Co(bipyridine)_3^{3+}$, or Fe-bleomycin.

The electrochemically-active moiety comprising the electrochemically-active reporter-labeled target molecule used in certain embodiments of the methods of the present invention is optionally linked to the target molecule through a linker, preferably having a length of from about 10 to about 20 Angstroms. The linker can be an organic moiety such as a hydrocarbon chain $(CH_2)_n$ (where n is an integer from 1 to about 20), or can comprise an ether, ester, carboxyamide, or thioether moiety, or a combination thereof. The linker can also be an inorganic moiety such as siloxane (O-Si-O). The length of the linker is selected so that the electrochemically-active moiety does not interfere with the molecular interaction to be detected.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

WHAT WE CLAIM IS:

1. An apparatus for electrical detection of molecular interactions between immobilized probe molecules and target molecules in a sample solution, comprising:

(a) a supporting substrate comprising an array of test sites,

(b) a plurality of porous, polymeric pads in contact with the supporting substrate at the test sites,

(c) a set of input electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each input electrode is arranged to address a subset of the test sites,

(d) a set of output electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each output electrode is arranged to address a subset of the test sites, and wherein each output electrode is in electrochemical contact with an input electrode,

(e) a plurality of linker moieties in contact with the porous, polymeric pads at the test sites,

(f) a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules,

(g) a means for producing an electrical signal at each input electrode,

(h) a means for detecting changes in the electrical signal at each output electrode, and

(i) an electrolyte solution in contact with the porous polymeric pads, input electrodes, output electrodes, linker moieties, and probe molecules,

wherein molecular interactions between the immobilized probe molecules and target molecules are detected as a difference in the electrical signal detected at each output electrode in the presence and absence of target molecules.

2. An apparatus for electrical or electrochemical detection of molecular interactions between immobilized probe molecules and target molecules in a sample solution, comprising:

(a) a supporting substrate comprising an array of test sites,

(b) a plurality of porous, polymeric pads in contact with the supporting substrate at the test sites,

(c) a set of input electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each input electrode is arranged to address a subset of the test sites,

(d) a set of output electrodes in contact with the plurality of porous, polymeric pads at the test sites, wherein each output electrode is arranged to address a subset of the test sites, and wherein each output electrode is in electrochemical contact with an input electrode,

(e) a plurality of linker moieties in contact with the porous, polymeric pads at the test sites,

(f) a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules,

(g) at least one reference electrode in electrochemical contact with the input and output electrodes,

(h) a means for producing an electrical signal at each input electrode,

(i) a means for detecting changes in the electrical signal at each output electrode, and

(j) an electrolyte solution in contact with the porous polymeric pads, input electrodes, output electrodes, linker moieties, reference electrode, and probe molecules,

wherein molecular interactions between the immobilized probe molecules and target molecules are detected as a difference in the electrical signal detected at each output electrode in the presence and absence of target molecules.

3. An apparatus for electrical detection of molecular interactions between immobilized probe molecules and target molecules in a sample solution, comprising:

(a) a supporting substrate comprising an array of test sites,

(b) a set of input electrodes in contact with the supporting substrate, wherein each input electrode is arranged to address a subset of the test sites,

(c) a set of output electrodes in contact with the supporting substrate at the test sites, wherein each output electrode is arranged to address a subset of the test sites, each output electrode is in electrochemical contact with an input electrode, and the output electrodes and input electrodes are interdigitated at the test site,

(d) a plurality of linker moieties in contact with either the input electrodes, the output electrodes, or both the input electrodes and output electrodes at the test sites,

(e) a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules,

(f) a means for producing an electrical signal at each input electrode,

(h) a means for detecting changes in the electrical signal at each output electrode, and

(i) an electrolyte solution in contact with the input electrodes, output electrodes, linker moieties, and probe molecules,

wherein molecular interactions between the immobilized probe molecules and target molecules are detected as a difference in the electrical signal detected at each output electrode in the presence and absence of target molecules.

4. An apparatus for electrical or electrochemical detection of molecular interactions between immobilized probe molecules and target molecules in a sample solution, comprising:

(a) a supporting substrate comprising an array of test sites,

(b) a set of input electrodes in contact with the supporting substrate, wherein each input electrode is arranged to address a subset of the test sites,

(c) a set of output electrodes in contact with the supporting substrate at the test sites, wherein each output electrode is arranged to address a subset of the test sites, each output electrode is in electrochemical contact with an input electrode, and the output electrodes and input electrodes are interdigitated at the test site,

(d) a plurality of linker moieties in contact with either the input electrodes, the output electrodes, or both the input electrodes and output electrodes at the test sites,

(e) a plurality of probe molecules immobilized to the linker moieties, wherein said probe molecules specifically bind to or interact with target molecules,

(f) at least one reference electrode in electrochemical contact with the input and output electrodes,

(g) a means for producing an electrical signal at each input electrode,

(h) a means for detecting changes in the electrical signal at each output electrode, and

(i) an electrolyte solution in contact with the input electrodes, output electrodes, linker moieties, reference electrode, and probe molecules,

wherein molecular interactions between the immobilized probe molecules and target molecules are detected as a difference in the electrical signal detected at each output electrode in the presence and absence of target molecules.

5. The apparatus of any of Claims 1, 2, 3, or 4, wherein the supporting substrate comprises ceramic, glass, silicon, silicon nitride, fabric, rubber, plastic, printed circuit board, compound semiconductors, or combination thereof.

6. The apparatus of either Claims 1 or 2, wherein the porous, polymeric pads comprise polyacrylamide gel, agarose gel, polyethylene glycol, cellulose gel, sol gel, polypyrrole, carbon, carbides, oxides, nitrides, or combination thereof.

7. The apparatus of Claim 6, wherein the porous, polymeric pads comprise polyacrylamide gel.

8. The apparatus of any of Claims 1, 2, 3, or 4, wherein the input electrodes comprise solid or porous gold, silver, platinum, copper, titanium, chromium, or aluminum, or metal oxide, metal nitride, metal carbide, carbon, graphite, conductive plastic, metal impregnated polymers, or combinations thereof.

9. The apparatus of Claim 8, wherein the input electrodes comprise platinum.

10. The apparatus of Claim 8, wherein the input electrodes comprise gold.

11. The apparatus of any of Claims 1, 2, 3, or 4, wherein the input electrodes comprise a conductive material and an insulating material.

12. The apparatus of Claim 11, wherein the conductive material is solid or porous gold, silver, platinum, copper, titanium, chromium, or aluminum, or metal oxide, metal nitride, metal carbide, carbon, graphite, conductive plastic, metal impregnated polymers, or combinations thereof.

13. The apparatus of Claim 12, wherein the conductive material is platinum.

14. The apparatus of Claim 12, wherein the conductive material is gold.

15. The apparatus of Claim 11, wherein the insulating material is glass, silicon, plastic, rubber, fabric, ceramic, printed circuit board, or combinations thereof.

16. The apparatus of Claim 15, wherein the insulating material is silicon.

17. The apparatus of Claim 15, wherein the insulating material is glass.

18. The apparatus of Claim 11, wherein the conductive material is embedded in the supporting substrate and the supporting substrate comprises the insulating material.

19. The apparatus of either Claims 1 or 2, wherein a portion of each input electrode is embedded in the porous, polymeric pads at the test sites addressed by said input electrode.

20. The apparatus of any of Claims 1, 2, 3, or 4, wherein the output electrodes comprises solid or porous gold, silver, platinum, copper, titanium, chromium, or aluminum, or metal oxide, metal nitride, metal carbide, carbon, graphite, conductive plastic, metal impregnated polymers, or combinations thereof.

21. The apparatus of Claim 20, wherein the output electrode comprises platinum.

22. The apparatus of Claim 20, wherein the output electrode comprises gold.

23. The apparatus of any of Claims 1, 2, 3, or 4, wherein the output electrode comprises a conductive material and an insulating material.

24. The apparatus of Claim 23, wherein the conductive material is solid or porous gold, silver, platinum, copper, titanium, chromium, or aluminum, or metal oxide, metal nitride, metal carbide, carbon, graphite, conductive plastic, metal impregnated polymers, or combinations thereof.

25. The apparatus of Claim 24, wherein the conductive material is platinum.

26. The apparatus of Claim 24, wherein the conductive material is gold.

27. The apparatus of Claim 23, wherein the insulating material is glass, silicon, plastic, rubber, fabric, ceramic, printed circuit board, or combinations thereof.

28. The apparatus of Claim 27, wherein the insulating material is silicon.

29. The apparatus of Claim 27, wherein the insulating material is glass.

30. The apparatus of Claim 23, wherein the conductive material is embedded in the supporting substrate and the supporting substrate comprises the insulating material.

31. The apparatus of either Claims 1 or 2, wherein a portion of each output electrode is embedded in the porous, polymeric pads at the test sites addressed by said output electrode.

32. The apparatus of Claim 31, wherein the output electrodes and input electrodes are interdigitated at the test site.

33. The apparatus of any of Claims 1, 2, 3, or 4, wherein the linker moieties comprise a conjugated polymer or copolymer film.

34. The apparatus of Claim 33, wherein the conjugated polymer or copolymer film is polypyrrole, polythiphene, polyaniline, polyfuran, polypyridine, polycarbazole, polyphenylene, poly(phenylenvinylene), polyfluorene, or polyindole, or their derivatives, copolymers, or combinations thereof.

35. The apparatus of any of Claims 1, 2, 3, or 4, wherein the linker moieties comprise a neutral pyrrole matrix.

36. The apparatus of any of Claims 1, 2, 3, or 4, wherein the linker moieties comprise thiol linkers.

37. The apparatus of any of Claims 1, 2, 3, or 4, wherein the probe molecules are oligonucleotides or nucleic acids.

38. The apparatus of Claim 37, wherein the probe molecules are aptamers.

39. The apparatus of any of Claims 1, 2, 3, or 4, wherein the probe molecules are proteins or peptides.

40. The apparatus of Claim 39, wherein the peptides are antibodies.

41. The apparatus of Claim 40, wherein the antibodies are a polyclonal antisera, polyclonal antibodies, or F(ab), F(ab)', $F(ab)_2$,or F_v fragments thereof.

42. The apparatus of Claim 40, wherein the antibodies are monoclonal antibodies, or F(ab), F(ab)', $F(ab)_2$,or F_v fragments thereof.

43. The apparatus of Claim 40, wherein the antibodies are F(ab) fragments or single-chain F_v fragments produced by *in vitro* libraries.

44. The apparatus of any of Claims 1, 2, 3, or 4, wherein the probe molecules comprise a natural products library, a phage display library, or a combinatorial library.

45. The apparatus of any of Claims 1, 2, 3, or 4, wherein the linker moieties comprise streptavidin and the probe molecules are biotinylated.

46. The apparatus of either Claims 1 or 2, wherein the probe molecules are first covalently linked to the linker moieties and then the linker moieties are placed in contact with the porous, polymeric pads.

47. The apparatus of either Claims 3 or 4, wherein the probe molecules are first covalently linked to the linker moieties and then the linker moieties are placed in contact with either the input electrodes, the output electrodes, or both the input electrodes and output electrodes.

48. The apparatus of either Claims 1 or 2, wherein the probe molecules are first covalently linked to the linker moieties, the linker moieties are mixed with porous, polymeric pad constituents, and then the porous, polymeric pads are polymerized.

49. The apparatus of either Claims 2 or 4, wherein the reference electrode comprises solid or porous gold, silver, platinum, copper, titanium, chromium, or aluminum, or metal oxide, metal nitride, metal carbide, carbon, graphite, conductive plastic, metal impregnated polymers, or combinations thereof.

50. The apparatus of Claim 49, wherein the reference electrode comprises platinum.

51. The apparatus of Claim 49, wherein the reference electrode comprises gold.

52. The apparatus of either Claims 2 or 4, wherein the conductive material is silver/silver chloride.

53. The apparatus of either Claims 2 or 4, wherein the reference electrode comprises a conductive material and an insulating material.

54. The apparatus of Claim 53, wherein the conductive material is solid or porous gold, silver, platinum, copper, titanium, chromium, or aluminum, or metal oxide, metal nitride, metal carbide, carbon, graphite, conductive plastic, metal impregnated polymers, or combinations thereof.

55. The apparatus of Claim 54, wherein the conductive material is platinum.

56. The apparatus of Claim 54, wherein the conductive material is gold.

57. The apparatus of Claim 53, wherein the insulating material is glass, silicon, plastic, rubber, fabric, ceramic, printed circuit board, or combinations thereof.

58. The apparatus of Claim 57, wherein the insulating material is silicon.

59. The apparatus of Claim 57, wherein the insulating material is glass.

60. The apparatus of Claim 53, wherein the conductive material is embedded in the supporting substrate and the supporting substrate comprises the insulating material.

61. The apparatus of any of Claims 1, 2, 3, or 4, wherein the supporting substrate further comprises a plurality of wells wherein each well encompasses a porous, polymeric pad, wherein a plurality of probe molecules is immobilized to linker moieties that are in contact with the porous, polymeric pad; an input electrode; and an output electrode.

62. The apparatus of any of Claims 1, 2, 3, or 4, wherein the means for producing an electrical signal at each input electrode comprises a multiplexer.

63. The apparatus of any of Claims 1, 2, 3, or 4, wherein the means for detecting changes in the electrical signal at each output electrode comprises a demultiplexer.

64. A method for the electrical detection of molecular interactions between a probe molecule immobilized at a specific test site in the apparatus of any of Claims 1, 2, 3, or 4 and a target molecule in a sample solution, comprising:

(a) applying a first electrical signal at an input electrode in contact with a first set of porous, polymeric pads, wherein the first set of porous, polymeric pads comprises the porous, polymeric pad at the specific test site,

(b) detecting the first electrical signal at an output electrode in contact with a second set of porous, polymeric pads, wherein the second set of porous, polymeric pads comprises the porous, polymeric pad at the specific test site,

(c) exposing the first and second sets of porous, polymeric pads to a sample mixture containing the target molecule,

(d) applying a second electrical signal at an input electrode in contact with the first set of porous, polymeric pads,

(e) detecting the second electrical signal at an output electrode in contact with the second set of porous, polymeric pads,

(f) comparing the first electrical signal detected in step (b) with the second electrical signal detected in step (e), and

(g) determining whether the first electrical signal is different from the second electrical signal.

65. The method of Claim 64, wherein molecular interactions between probe molecules and target molecules are detected by using an electrical or electrochemical detection method selected from the group consisting of impedance spectroscopy, cyclic voltammetry, AC voltammetry, pulse voltammetry, square wave voltammetry, AC voltammetry, hydrodynamic modulation voltammetry, conductance, potential step method, potentiometric measurements, amperometric measurements, current step method, other steady-state or transient measurement methods, and combinations thereof.

66. The method of Claim 64, wherein molecular interactions between probe molecules and target molecules are detected by using an electrical or electrochemical detection method that is AC impedance and the AC impedance is measured over a range of frequencies.

67. The method of Claim 64, wherein molecular interactions between probe molecules and target molecules are detected by using an electrical or electrochemical detection method that is AC impedance and the AC impedance is measured by transient methods with AC signal perturbation superimposed upon a DC potential applied to an electrochemical cell.

68. The method of Claim 64, wherein molecular interactions between probe molecules and target molecules are detected by using an electrical or electrochemical detection method that is AC impedance and the AC impedance is measured by impedance analyzer, lock-in amplifier, AC bridge, AC voltammetry, or combinations thereof.

69. The method of Claim 64, wherein the target molecules are labeled with an electrochemically-active reporter molecule prior to exposing the first and second sets of porous, polymeric pads to a sample mixture containing the target molecule.

70. The method of Claim 69, wherein the electrochemically-active reporter-molecule comprises a transition metal complex.

71. The method of Claim 70, wherein the transition metal complex further comprises a transition metal ion that is ruthenium, cobalt, iron, zinc, nickel, magnesium, or osmium.

72. The method of Claim 70, wherein the electrochemically-active reporter-labeled target molecules are labeled with electrochemical reporter groups selected from the group consisting of 1,4-benzoquinone, ferrocene, tetracyanoquinodimethane, N,N,N',N'-tetramethyl-p-phenylenediamine, and tetrathiafulvalene.

73. The method of Claim 70, wherein the electrochemically-active reporter-labeled target molecules are labeled with electrochemical reporter groups selected from the group consisting of 9-aminoacridine, acridine orange, aclarubicin, daunomycin, doxorubicin, pirarubicin, ethidium bromide, ethidium monoazide, chlortetracycline, tetracycline, minocycline, Hoechst 33258, Hoechst 33342, 7-aminoactinomycin D, Chromomycin A_3, mithramycin A, Vinblastine, Rifampicin, $Os(bipyridine)_2(dipyridophenazine)_2^{+}$, $Co(bipyridine)_3^{3+}$, and Fe-bleomycin.

74. The method of Claim 64, wherein the first and second electrical signals are applied using a multiplexer.

75. The apparatus of Claim 64, wherein the first and second electrical signals are detected using a demultiplexer.

ABSTRACT OF THE DISCLOSURE

The present invention provides an apparatus and methods for the electrical or electrochemical detection of molecular interactions between biological molecules. Specifically, the invention provides an apparatus for the electrical or electrochemical detection of molecular interactions between biological molecules that comprises a column-and-row addressable biochip array.

Figure 1

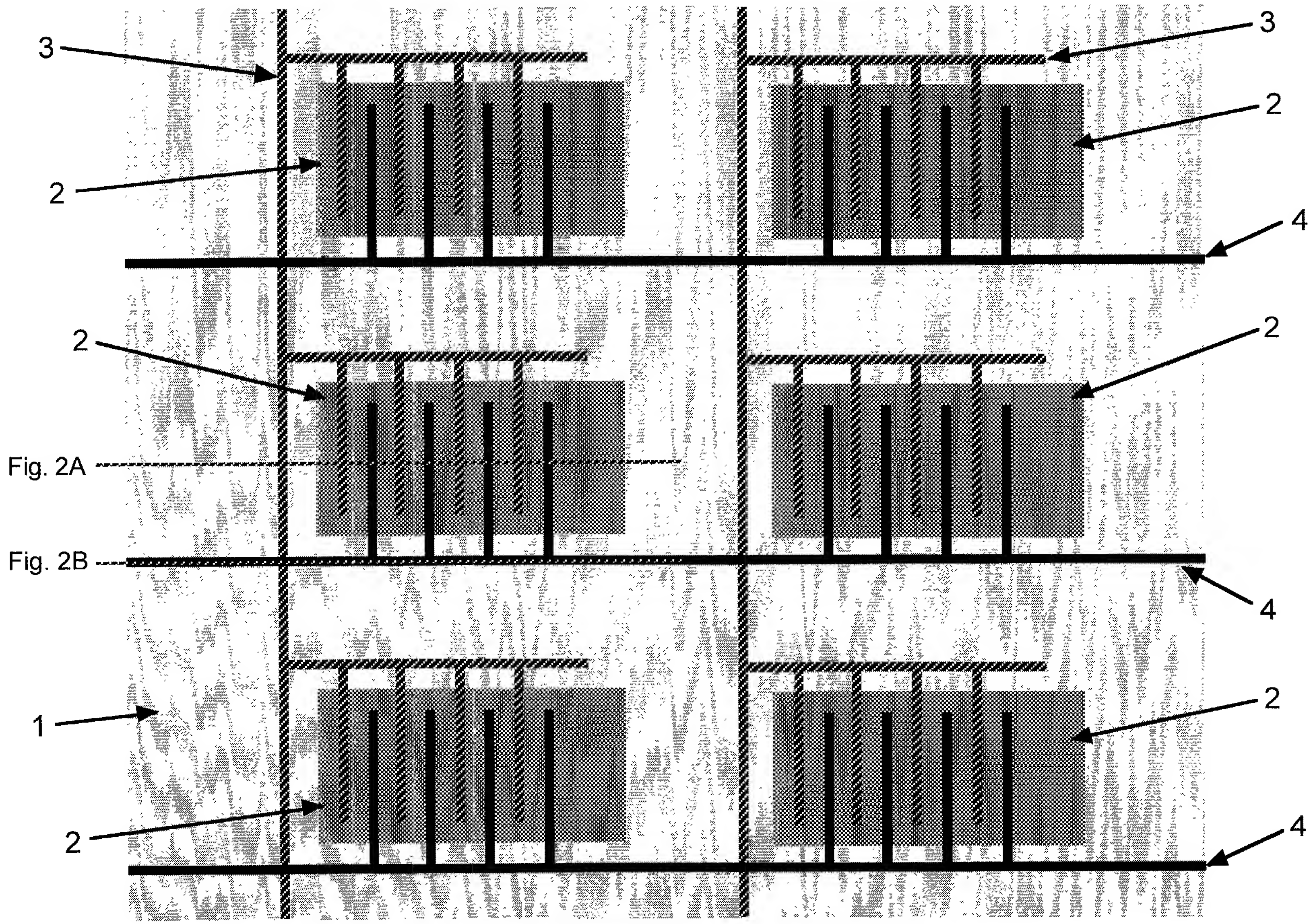


Figure 2A

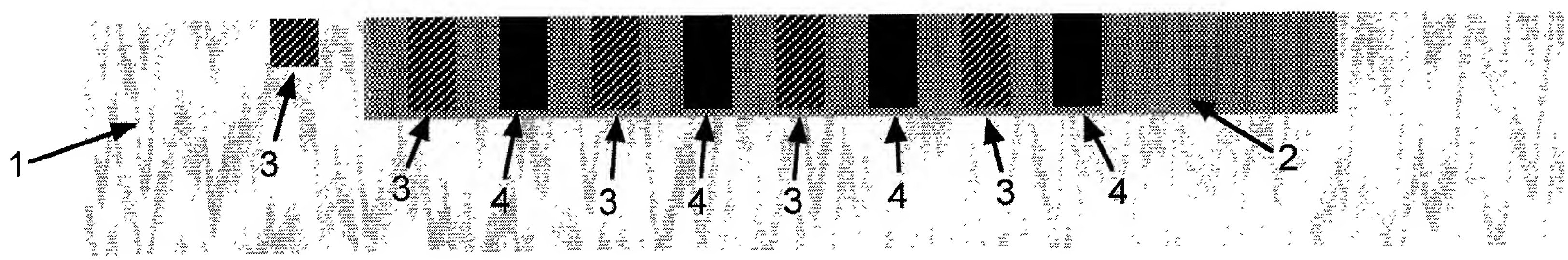


Figure 2B

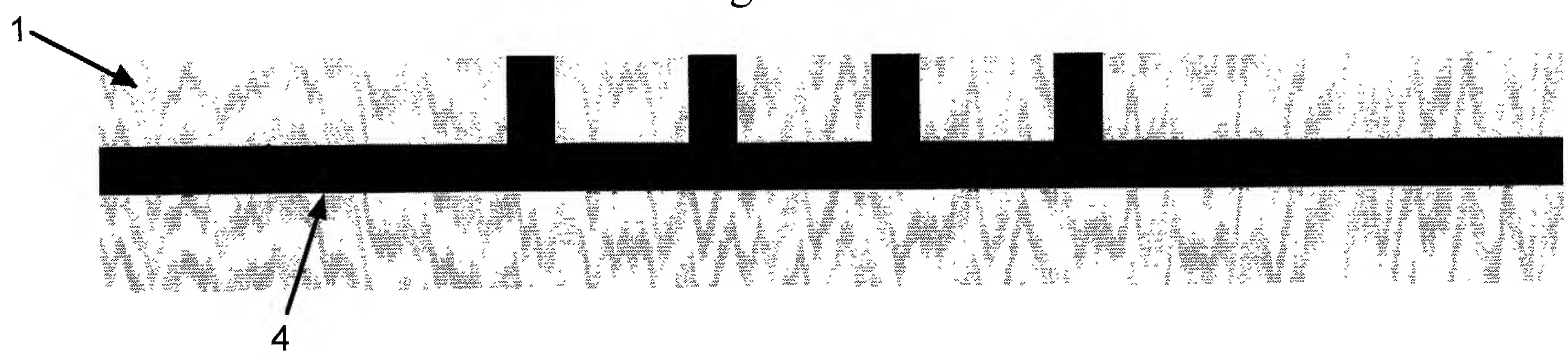


Figure 3

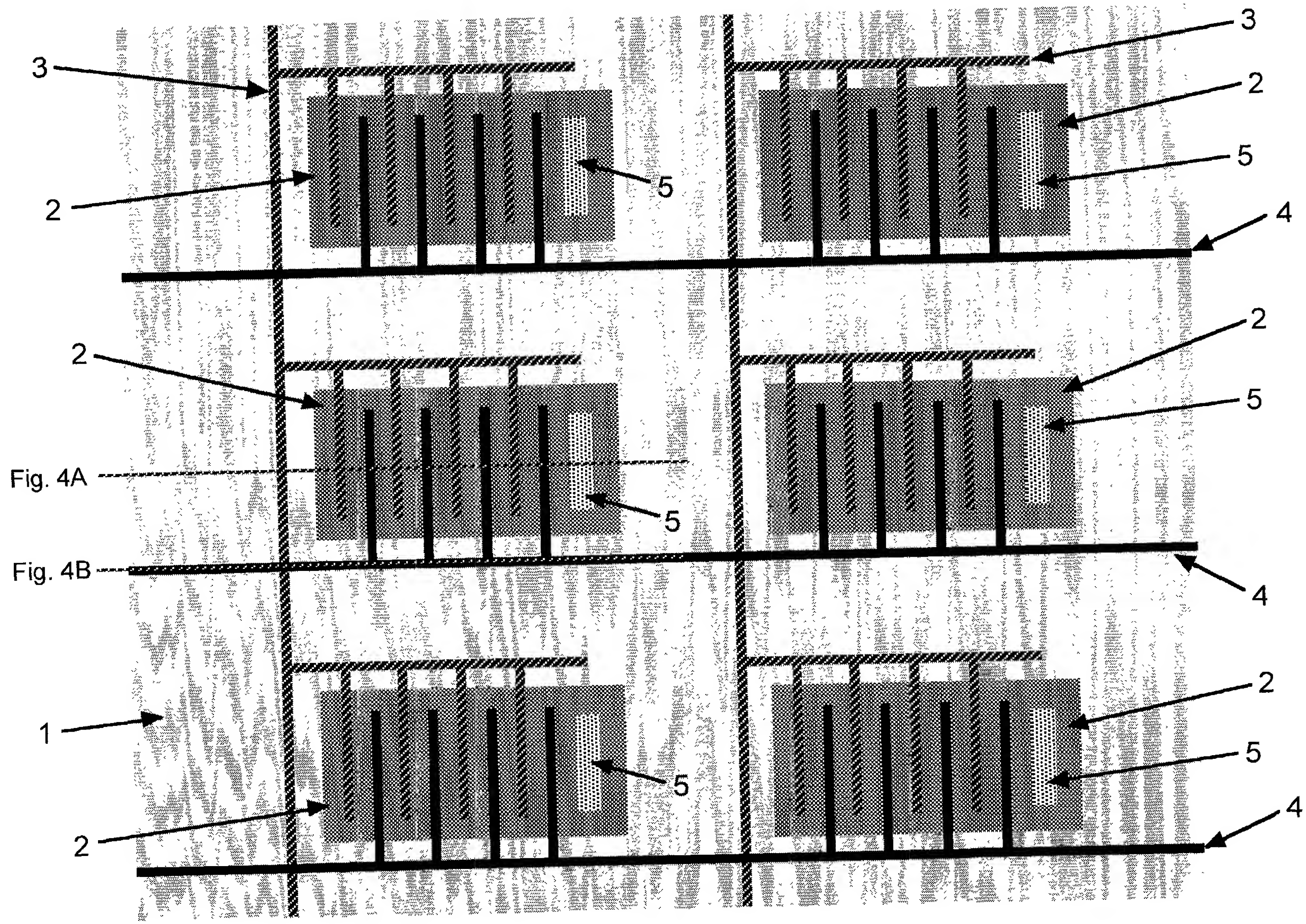


Figure 4A

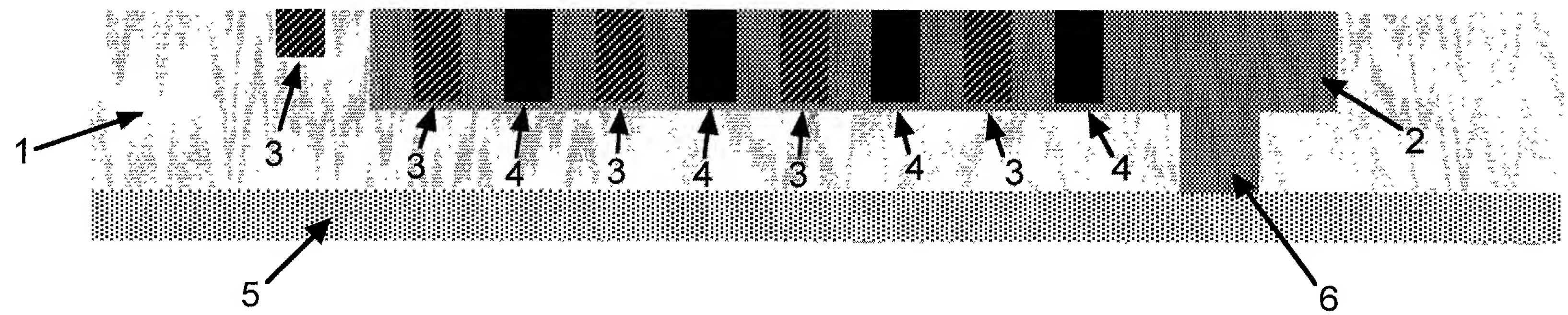
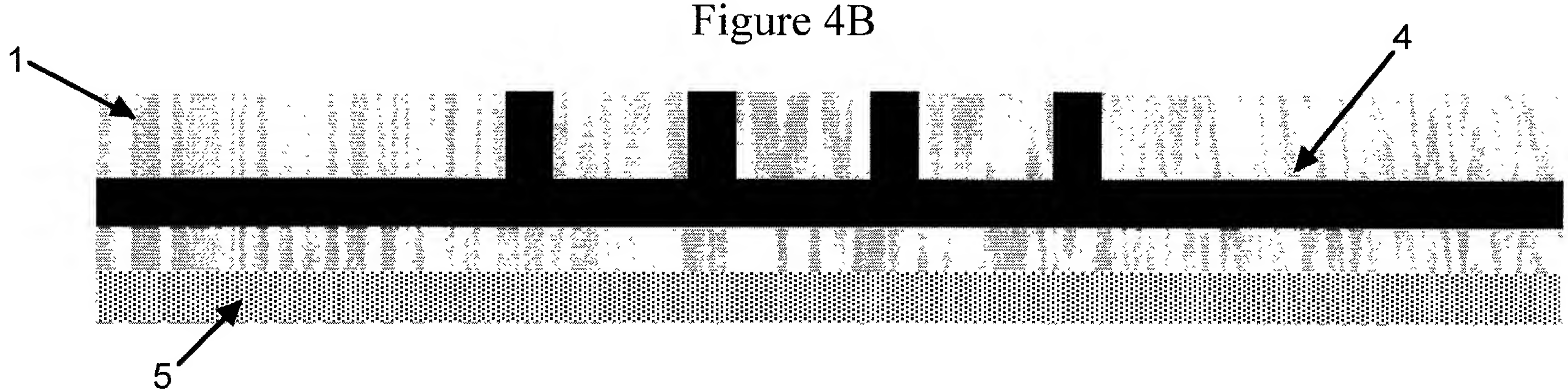


Figure 4B



Case No.: MBHB00-025

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Addressable Array for High Density Electrical and Electrochemical Detection of Biomolecules

the specification of which is attached hereto unless the following space is checked:

☐ was filed on June 9, 2000 as United States Application Serial Number .

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

	Number	Country	Day/Month/Year Filed
1.			
2.			

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

	Application Number	Filing Date
1.		

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

	Application Number	Filing Date	Status: patented, pending, abandoned
1.			
2.			

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I hereby appoint the following attorneys and agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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